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RECOMBINANT DNA RESEARCH

Proposed Revised Guidelines

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On June 23, 1976, the Director, National Institutes of Health (NIH), with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, issued guidelines that govern the conduct of NIH-supported research involving recombinant DNA molecules. These guidelines appeared in the FEDERAL REGISTER, July 7, 1976 (41 FR 27902 et seq.). The purpose of this notice is to report developments since that publication and to invite public comment on the following draft proposing revised guidelines.

In recombinant DNA experiments, "genes"—deoxyribonucleic acid (DNA) molecules—from living organisms can be transferred to single cells from completely unrelated organisms. These experiments depend on the ability to join genetic material from different sources and to propagate the resulting elements in single bacterial and animal cells.

The new recombinant DNA technique has resulted in a profound and qualitative change in the field of gentics. Developments in genetic research, particularly in the last 4 years, open avenues to science that were previously inaccessible. Hypotheses and ideas that were not testable can now be rigorously investigated. Understanding of basic biological phenomena has already been enhanced, and the promise of recombinant DNA research for better understanding and improved treatment of human disease is great. Further experimental data will be required to delimit the benefits that may be derived through this techniques.

There is also a possible risk that microorganisms with foreign genes could cause disease or adversely affect the environment if they should escape from the laboratory and infect human beings, animals, or plants. Many recombinant DNA experiments have been conducted throughout the world during the past 5 years and are continuing. To date, no known hazardous organism has been produced in this work. Thus, the risk of converting harmless organisms to harmful ones by recombinant DNA experiments remains hypothetical. Further work will eventually determine the limits of these hypothetical risks.

Approximately 3 years ago, because of the perceived potential hazards, scientists conducting recombinant DNA research called for a moratorium on certain experiments pending an assessment of risk and the development of appropriate guidelines. These scientists requested NIH to create an advisory committee to develop such guidelines.

In December 1975 the NIH Recombinant DNA Molecule Program Advisory Committee (or simply Recombinant Advisory Committee) proposed guidelines to the Director of NIH. The guidelines were reviewed at a public hearing before the Advisory Committee to the Director, NIH, in Februray 1976. After a review of all public comments, NIH Guidelines were rleeased on June 23, 1976, accompanied by a Director's Decision addressing the issues raised at the public hearing and in subsequent correspondence. The Guidelines set strict conditions for the conduct of NIH-supported research in this area. They prohibit certain types of experiments and require special safety conditions for other types. The provisions are designed to afford protection with a wide margin of safety to workers, the public, and the environment. On July 7, 1976, the NIH Guidelines were published in the FEDERAL REGISTER, and on September 9, 1976, a Draft Environmental Impact Statement on the Guidelines was similarly presented for public comment (41 FR 38425 et seq.).

A Federal Interagency Committee on Recombinant DNA Research recommended in March 1977 that legislation be passed to extend the standards of the NIH Guidelines to all recombinant DNA activities in the public and private sectors. On the basis of the recommendations, Health, Education, and Welfare Secretary Joseph A. Califano, Jr., had legislation developed, and an Administration bill was introduced in Congress. Congressional hearings were held and seperate bills are now being considered by the House of Representatives and the Senate to convert the Guidelines to standards for regulatory purposes. It should be noted that certain types of experiments now covered in the Guidelines would no longer be subject to regulation in the bills.

On April 11, 1957, the Secretary, DHEW, adopted as official policy for DHEW the "NIH Guidelines for Research Involving Recombinant DNA Molecules" (42 FR 40485). The NIH Guidelines, as released in 1976, have governed all NIH supported or conducted recombinant DNA research. From discussions relating to the release of the NIH Guidleines, it is recognized that there is need for a continual updating in light of new knowledge. In 1977 the Recombinant Advisory Committee, which had proposed the original guidelines, began the process of revising the current NIH Guidelines on the basis of information accumulated on the effectiveness of physical and biological containment and on the biology of the hosts and vectors utilized in recombinant DNA research. A Subcommittee to the full Committee held open meetings in March and April to consider comments received since the NIH Guidelines were released. On the basis of these deliberations, revisions were proposed and were considered by the full Committee at public meetings in May and June. On September 1, 1977, the proposed revised Guidelines were referred to the Director, NIH, for final decision.

The proposed revised Guidelines will be published in September for comment in the "NIH Recombinant DNA Technical Bulletin." The Bulletin is a new NIH publication that will attempt to link investigators involved in recombinant DNA research both in the United States and abroad with the advisory groups and organizations active in this area.

To provide further opportunity for public comment and consideration, the proposed revised Guidelines are offered below. All comments received will be considered by the Director, NIH, and by the Advisory Committee to the Director, NIH, at its December meeting. On the basis of the comments received and review by the Advisory Committee, the Director will subsequently issue final Guidelines accompanied by a decision document explaining the modifications.

Please address any comments on these draft policies and procedures to the Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md. 20014. All comments should be received by November 15, 1977. Additional copies of this notice are available from:

Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md. 20014.

Dated: September 16, 1977.

DONALD S. FREDRICKSON,
Director,
National Institutes of Health.

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I. INTRODUCTION

The purposes of these Guidelines is to establish procedures for handling organisms and viruses containing recombinant DNA molecules. Recombinant DNA molecules are defined as molecules consisting of segments of DNA from different genomes which have been joined end-to-end outside living cells and have the capacity to infect some host cell and be maintained therein. These Guidelines, however, pertain only to "novel recombinant DNAs," here defined as molecules that consist of segments of any DNA from different species that are not known to exchange chromosomal DNA by natural physiological processes. The Director of NIH with the advice of the Recombinant DNA Molecule Program Advisory Committee shall prepare the list of those combinations of DNAs (viral, extrachromosomal or chromosomal) which are not considered novel for this purpose and are, therefore, not covered by these Guidelines. In general, recombinant

DNA molecules formed from any combination of DNAs will not be considered novel when all the components are derived from genomes known to replicate within the organism used to propagate the recombinant DNA.[1]

A major task of this Committee has been first to prepare and then periodically to revise Guidelines that will allow the promise of recombinant DNA research to be realized, while providing the caution that is dictated by concerns about the possibility of hazard, however remote.

The present revisions take into account many communications from both scientists and non-scientists since the original publication of the Guidelines. During this period the Committee has also become better informed about the general ecology and epidemiology of infectious microorganisms. Of particular relevance has been the information received from many medical microbiologists, including data from experiments with *Escherichia coli* K-12. These experiments include a demonstration that strain K-12 cannot be made pathogenic even when provided, by standard genetic techniques, with the genes for known toxins and other pathogenic properties. Other relevant experiments that have been reported show that the incorporation of foreign DNA does not increase, but rather tends to decrease, the general fitness of microorganisms; this phenomenon further contributes to the unlikelihood that cells carrying recombinant DNA will survive in nature. Indeed, everything we have learned tends to diminish our estimate of the risk associated with recombinant DNA in *E. coli* K-12. Nevertheless, the revised Guidelines continue to be deliberately restrictive, with the intent of erring on the side of caution.

In constructing these Guidelines it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and study sections recommend changes in the Guidelines to permit their use.

It is urged that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures employed, to aid others who might consider repeating this work.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs (1-14). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated bio-hazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the Guidelines for research on the different kinds of recombinant DNAs (Section III).

A. *Standard practices and training.* The first principle of containment is a strict adherence to good microbiological practices (1-10). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aseptic techniques and instruction in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

B. *Physical containment levels.*—The objective physical containment is to confine organisms containing novel recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing novel recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. For example the "Classification of Etiologic Agents on the Basis of Hazard" (2), prepared by the Center for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels (3).

It is recognized that other combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Recombinant DNA Molecule Program Advisory Committee to other combinations which achieve an equivalent level of containment. Additional material on physical containment for plant host-vector systems is found on page III-24.

1. *P1 level.*—a. *Laboratory practices.* (1) Laboratory doors shall be kept closed while experiments are in progress.

(2) Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

(3) All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal.

(4) Although pipetting by mouth is permitted, it is recommended that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed.

(5) Eating, drinking, smoking, and storage of foods are not permitted in the working area.

(6) Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

(7) Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

(8) Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leakproof container which is closed before removal from the laboratory.

(9) An insect and rodent control program shall be instituted.

(10) The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

(11) Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

b. *Containment equipment.* Special containment equipment is not required at the P1 level.

c. *Special laboratory design.* Special laboratory design is not required at the P1 level.

2. *P2 level.*—a. *Laboratory practices.* (1) Laboratory doors shall be kept closed while experiments are in progress.

(2) Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

(3) All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal.

*(4) Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

(5) Eating, drinking, smoking, and storage of food are not permitted in the laboratory area.

(6) Persons shall wash their hands after handling organisms containing recombinant

*Denotes laboratory practices, containment equipment, or special laboratory design which were not required at the next lower level of containment.

DNA molecules and when they leave the laboratory.

*(7) Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a *hot* inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

(8) Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leakproof container which is closed before removal from the laboratory.

*(9) Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

*(10) Children under 12 years of age shall not enter the laboratory.

*(11) The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

(12) An insect and rodent control program shall be instituted.

*(13) The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

*(14) Animals not related to the experiment shall not be permitted in the laboratory.

*(15) Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

*(16) The laboratory shall be kept neat, clean, and free of materials not pertinent to the research.

*(17) Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

b. *Containment equipment.* *Biological safety cabinets[3] shall be used to contain aerosol-producing equipment such as blenders, lyophilizers, sonicators, and centrifuges when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

c. *Special laboratory design.* *An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

3. *P3 level.*—a. *Laboratory practices.* (1) doors shall be kept closed while experiments are in progress.

*(2) Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

(3) All biological wastes shall be decontaminated before disposal. Other contaminate materials such as glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal.

(4) Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

(5) Eating, drinking, smoking, and storage of food are not permitted in the laboratory area.

(6) Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

(7) Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

(8) Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leakproof container which is closed before removal from the laboratory.

*(9) Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

(10) Children under 12 years of age shall not enter the laboratory.

*(11) The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3 level containment are in progress. Freezers and refrigerators used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

(12) An insect and rodent control program shall be instituted.

*(13) Laboratory clothing that protects street clothing (i.e., long sleeve solid-front or wrap-around gowns, no-button or slip-over jackets, etc.) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

*(14) Raincoats, overcoats, topcoats, coats, hats, caps, and such street out-wear shall not be kept in the laboratory.

*(15) Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately following the handling procedure and decontaminated.

*(16) Animals and plants not related to the experiment shall not be permitted in the laboratory.

*(17) Vacuum outlets shall be protected by filter and liquid disinfectant traps.

(18) Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

(19) The laboratory shall be kept neat, clean, and free of materials not pertinent to the research.

*(20) If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurently with experiments requiring P3 level physical containment, they shall be conducted in accordance with all P3 level laboratory practices.

b. *Containment Equipment.* *(1) Biological safety cabinets[3] shall be used for all equipment and manipulations that produce aerosols (e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging etc.) where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

*(2) Laboratory animals held in a P3 area shall be housed in partial containment caging systems such as Horsfall units, open cages placed in ventilated enclosures, solid wall and bottom cages covered by filter bonnets, or solid wall and bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors.

NOTE: Conventional caging systems may be used; *Provided,* That all personnel wear appropriate personal protective devices. These shall include at a minimum, wrap around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these personal protective devices are required.)

*(3) *Alternative selection of containment equipment.* Experimental procedures involving a host-vector system which provides a one-step higher level of biological containment than that specified in Section III can be conducted in the P3 laboratory using containment equipment requirements specified for the P2 level of physical containment. Experimental procedures involving a host-vector system which provides a one-step lower level of biological containment than that specified in Section III can be conducted in the P3 laboratory using containment equipment requirements specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I on the following page.

TABLE I.—*Combinations of containment safeguards*

Classification of experiment according to guidelines		Combinations of physical and biological containment			
Physical containment	Biological* containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV3	P3	P3	P3	HV3
P3	HV3	P3	P3	P4	HV2
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P2	HV3
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P2	P2	HV2

*See sec. II, D. for description of biological containment.

c. *Special Laboratory Design.* *(1) The laboratory shall be separated from areas which are open to unrestricted traffic flow within the building by a controlled access area. A controlled access area is an anteroom, a change room, an air lock or any other double door arrangement which separates the laboratory from areas which are open to unrestricted traffic flow.

*(2) The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

*(3) A foot, elbow, or automatically operated hand washing facility shall be provided near each primary laboratory exit area.

*(4) Windows in the laboratory shall be sealed.

*(5) Laboratory doors shall be self-closing.

(6) An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA colecules are used.

*(7) An exhaust air ventilation system shall be provided. This system shall be balanced so that the direction of air flow is

from the controlled access area into the laboratory environment. The exhaust air shall not be recirculated to any other areas of the building. Recirculation of air within the laboratory room, however, may be provided. The exhaust air from the laboratory shall be discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The exhaust air from the laboratory can be discharged to the outdoors without filtration or other treatment.

*(8) The treated exhaust air from Class I and Class II biological safety cabinets[3] may be discharged either directly to the laboratory or to the outdoors. The treated exhaust air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system as to avoid any interference with the air balance of the cabinet or building exhaust air system.

4. *P4 Level*—a. *Laboratory Practices.* (1) Laboratory doors shall be kept closed while experiments are in progress.

(2) Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

(3) All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal.

(4) Mechnical pipetting devices shall be used; pipetting by mouth is prohibited.

*(5) Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

(6) Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

(7) Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

*(8) Biological materials to be removed from the P4 facility in a viable or intact state, shall be transferred to a nonbreakable sealed contained which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

*(9) No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been sterilized or decontaminated as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility though a pass-through fumigation chamber.

*(10) Materials within the Class III cabinets shall be removed from the cabinet system only after being sterilized in an attached double-door autoclave or after being contained in a non-breakable seaaled container which is then passed through a disinfectant dunk tank or a fumigation chamber.

*(11) Only persons whose entry into the P4 facility is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the nature of the research being conducted and instructed as to the appropriate safeguards to ensure their safety before entry. Such persons shall comply with instructions and all other required procedures.

*(12) Persons under 18 years of age shall not enter the P4 facility.

*(13) Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each exit from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

*(14) Street clothing shall be removed in the outer side of the P4 facility clothing change area and kept there. Complete laboratory clothing including undergarments, pants and shirts or jumpsuits, shoes, head cover, and gloves shall be provided and used by all persons who enter into the P4 facility. Upon exit, this clothing shall be stored in lockers provided for this purpose or discarded into collection hampers before personnel enter into the shower area.

*(15) The universal biohazard sign is required on the P4 facility access doors and all interior doors to individual laboratory rooms where experiments are conducted.

(16) An insect and rodent control program shall be instituted.

(17) Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

(18) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

(19) The laboratory shall be kept neat, clean, and free of materials not pertinent to the research.

*(20) If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4 level containment, they shall be conducted in accordance with all P4 level laboratory practices specified in this section.

b. *Containment equipment.* *(1) Experimental procedures involving organisms which require P4 level physical containment shall be conducted either in (a) a Class III cabinet system or in (b) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure isolation suits.

*(2) Laboratory animals involved in experiments requiring P4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units, open cages placed in ventilated enclosures, or solid wall and bottom cages covered by filter bonnets, or solid wall and bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

*(3) *Alternative selection of containment equipment.* Experimental procedures involving a host-vector system which provides a one-step higher level of biological containment than that specified in Section III can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II on following page.

TABLE II.—*Combinations of containment safeguards*

Classification of experiment according to guidelines		Combinations of physical and biological containment			
Physical containment	Biological* containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	P4	P3	HV2

*See sec. II, D. for description of biological containment.

c. *Special laboratory design.* *(1) The laboratory shall be located in a restricted access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel egress is through the shower area to the change room. A double door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

* (2) Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell, which readily allows vapor phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces are sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor phase decontaminant under static pressure conditions; this requirement does not imply that these surfaces must be airtight.)

* (3) A foot, elbow, or automatically operated hand washing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in open-face biological safety cabinets.

*(4) Where a central vacuum system is provided, it shall not serve areas outside of the P4 facility. The vacuum system shall include in-line HEPA filters as near as practicable to each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply and liquid and gaseous services provided to the P4 facility shall be protected by devices that prevent back flow.

*(5) Food-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to the fountain shall be independent of the water services to the laboratory areas of the P4 facility.

(6) Laboratory doors shall be self-closing.

*(7) A double door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

*(8) A pass-through dunk tank or fumigation chamber shall be provided for removal of material and equipment that cannot be heat sterilized from the P4 facility.

* (9) All liquid affluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be inactivated by chemical treatment. HEPA filters shall be installed in all effluent drain vent lines.

*(10) An individual supply and exhaust air ventilation system shall be provided for the P4 facility. The system shall maintain pressure differentials and directional air flow as required to assure inflow from areas outside of the P4 facility toward areas of highest potential risk within the P4 facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

*(11) Recirculation of air within individual laboratories of the P4 facility is permissible provided that this air is filtered by a HEPA filter.

*(12) The exhaust air from the facility shall be filtered by HEPA filters and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow in situ decontamination before removal and to facilitate certification testing after replacement.

(13) The treated exhaust air from Class I and Class II biological safety cabinets[2] may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets or the facility exhaust air system.

* (14) A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life support system. The life support system shall be provided with alarms and emergency backup tank air. Entry to this area is through an air-lock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal. The exhaust air from the suit area shall be filtered by two sets of HEPA filters installed in series. A duplicate filtration unit and exhaust fan shall be provided. An emergency power source shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. Emergency lighting and communication systems shall be provided.

The internal shell of the suit area shall be airtight. A double door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

C. *Shipment.* Novel recombinant DNA when contained in an organism or virus, shall be shipped in compliance with the requirements issued by the U.S. Public Health Service (§ 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (§ 173.387(b) of Part 173, Title 49, Code of Federal Regulations), and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6–D) for shipment of etiologic agents (Appendix A).

The packaging and shipment of organisms and viruses containing recombinant DNA molecules shall be in compliance with all requirements specified in subparagraphs (1)–(5) of paragraph (c), "Transportation; etiologic agents subject to additional requirements.", of § 72.25 of Part 72, Title 42, Code of Federal Regulations. Subparagraph (6) of paragraph (c) of § 72.25 of Part 72, Title 42, Code of Federal Regulations shall apply to the shipment of all viable host and vector organisms which require P4 physical containment.

Additional information on packaging and shipment is given in Appendix A.

D. *Biological containment*—1. *Levels of biological containment.* In considering biological containment, the vector (plasmid or virus) for the recombinant DNA and the host (bacterial, plant or animal cell) in which the vector is propagated in the laboratory will be considered together. Any vector and host which are to provide biological containment must be constructed so that the following types of "escape" are minimized:

Survival of the vector in its host outside of the laboratory. Transmission of the vector from the propagation host to other non-laboratory hosts.

The following levels of biological containment (*Host-Vector Systems*) for prokaryotes will be established; specific criteria will depend on the organism to be used. Eukaryotic host-vector systems are considered in Section III.

a. *HV1.* A host-vector system which provides a moderate level of containment. The host should have a low potential for survival in its natural environment, and the vector should have a low potential for transmission of its recombinant DNA.

Specific systems—(1) *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColEl, or derivatives thereof (15–21)) and variants of bacteriophage, such as λ (22–27). The *E. coli* K–12 hosts should not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

(2) *Other prokaryotes.* Hosts and vectors should be, at a minimum, comparable in containment to *E. coli* K–12 with a nonconjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Section II, D. 2.).

b. *HV2.* These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions.

Specific systems—(1) For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host as a consequence of transmission of the cloned DNA fragment.

(2) For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

c. *HV3.* These are host-vector systems in which:

(1) All HV2 criteria are met.

(2) The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

(3) No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or unique laboratory controlled conditions or is blocked by the inserted DNA.

(4) The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

(5) The relevant genotypic and phenotypic traits of such HV3 systems have been independently confirmed.

This general description can be applied directly to the specific case of an EK3 system.

2. *Certification of Host-Vector Systems*—a. *Responsibility.* Recommendation to the Director of NIH for certification of HV1 systems other than *E. coli* K–12, and HV2 and HV3 host-vector systems, is the responsibility of the NIH Recombinant DNA Molecule Program Advisory Committee. Data on construction, properties, and testing of proposed host-vector systems will be analyzed and reviewed by a subcommittee composed of one or more members of the NIH Recombinant DNA Molecule Program Advisory Committee and other individuals chosen because of their expertise in evaluating such data. Such subcommittees shall provide a written report to the NIH Recombinant DNA Molecule Program Advisory Committee. The Committee will evaluate this report and any other available information at a regular meeting.

The NIH Recombinant DNA Molecule Program Advisory Committee can recommend that a certification be rescinded at any time, should new data or new considerations invalidate the previous decision. In such cases, investigators may be asked to transfer their cloned DNA into a different approved system.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently approved. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. More substantial modifications of a certified system may necessitate submission of complete testing data.

b. *Data to be submitted for certification*—(1) *HV1 systems other than E. Coli K–12.* The following types of data should be submitted, modified as appropriate for the particular system under consideration:

(i) A description of the organism and vector, the strain's natural habitat and growth requirements; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; any relevant information on its pathogenicity or toxicity.

(ii) A description of the history of the particular strains and vectors to be used; information on any mutations which render this organism less able to survive or transmit genetic information.

(iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

(2) *HV2 systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from the NIH Office of Recombinant DNA Activities concerning data to be submitted. In general, the following types of data are required:

(i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits.

(ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system.

(iii) Data on the survival of the host-vector system under non-permissive in vitro conditions designed to represent the natural environment.

(iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions.

(v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation.

(vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments.

Data must be submitted in writing to the NIH Office of Recombinant DNA Activities. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the Recombinant DNA Molecule Program Advisory Committee. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the NIH Recombinant DNA Molecule Program Advisory Committee and its subcommittee.

(3) *HV3 systems.* Putative HV3 systems will, as the first step in certification, be certified as HV2 systems. Systems which meet criteria (1), (2), and (3) for HV3 (See page II-34) will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selected by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated; tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

3. *Distribution of certified host-vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates.

If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotypic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH on recommendation of the Recombinant DNA Molecule Program Advisory Committee; and (iv) remind the user of responsibility for notifying the NIH Office of Recombinant DNA Activities of any discrepancies with the reported properties or any problems in the safe use of the system.

III. Experimental Guidelines

As a general rule, the level of containment required for experiments with novel recombinant DNA shall not be less than that required for handling the most hazardous component, i.e., vector, host, or inserted DNA. Handling the purified DNA will require only minimal precautions.

The above rule by itself effectively precludes certain experiments—namely, those in which one of the components is in Class 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (See Appendix B), as these are excluded from the United States by law and USDA administrative policy. There are additional experiments which are not to be performed at this time. These are considered prior to presentation of the containment guidelines for permissible experiments.

A. *Experiments that are not to be performed.* We recognize that certain of the recombinants placed in this category might pose no biohazard and could be adequately contained at this time. Nonetheless, it is prudent to defer experiments on these recombinant DNAs until there is more information to assess accurately the danger or lack thereof, and to allow the construction of more effective biological barriers. In this respect, these Guidelines probably are more stringent than those that ultimately will be necessary.

The following experiments are not to be initiated at the present time: (i) Cloning of recombinant DNAs derived from the pathogenic organisms in Classes 3, 4, and 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (See Appendix B), or oncogenic viruses classified by NCI as moderate risk (See Appendix B), or cells known to be infected with such agents, regardless of the host-vector system used. (ii) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of potent toxins (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.). (iii) Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic enchange. (iv) Deliberate release into the environment of any organism containing novel recombinant DNA. (v) Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. (vi) Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing novel recombinant DNAs, unless the recombinant DNAs are rigorously characterized and are shown to be free of harmful genes.[3] We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

Specific experiments in these categories may be excepted from these rules: *Provided,* That these experiments are expressly approved by the Director, NIH, on recommendation of the Recombinant DNA Molecule Program Advisory Committee. In making such exceptions, weight will be given both to scientific and societal benefits and to potential risks.

B. *Containment Guidelines For Permissible Experiments.*—1. *Classification Of Experiments Using The E. coli K-12 Host-Vector Systems.*—Most recombinant DNA experiments currently being done employ *E. coli* K-12 host-vector systems. These are also the systems for which we have the most experience and knowledge (i) regarding the effectiveness of biological containment provided by existing hosts and vectors, and (ii) necessary for the construction of more effective biological barriers.

We therefore consider DNA recombinants in *E. coli* K-12 before proceeding to other host-vector systems.

It has been necessary, throughout this section, to use words and phrases such as "purified" or "rigorously characterized." In the text such terms are marked with footnote reference numbers. These footnotes (Section V) define more fully what is intended by the use of these terms.

In the following classification of containment criteria for different kinds of novel recombinant DNAs, the stated levels of physical and biological containment are minimum levels. The use of higher levels of biological containment (EK3>EK2>EK1) is encouraged if they are available and are equally appropriate for the purposes of the experiment.

a. *Shotgun experiments.* These experiments involve the production of novel recombinant DNAs between the vector and portions of the total DNA or (preferably) any partially purified fraction thereof from the specified cellular source. Care should be taken either to preclude or eliminate contaminating microorganisms before isolation of the DNA.

(1) *Eukaryotic DNA Recombinants.*—(a) Primates. P3 physical containment+an EK2 host-vector for DNA from uninfected (i.e., not productively infected) cells; otherwise, P4+EK1 or P3+EK3 must be used.

(b) *Other mammals.* P2 physical containment+an EK2 host-vector.

(c) *Birds.* P2 physical containment+an EK2 host-vector.

(d) *Cold-Blooded Vertebrates.* P2 physical containment+an EK2 host-vector or P3+EK1, except for uncontaminated embryonic or germline DNA from laboratory-reared animals which requires P2+EK1 or P1+EK2. If the eukaryote is known to produce a potent polypeptide toxin,[4] the containment shall be increased to P3+EK2.

(e) *Other Cold-Blooded Animals And Lower Eukaryotes.* This large class of eukaryotes is divided into two groups:

1. Species that are known to produce a potent polypeptide toxin [4] for vertebrates, or are known pathogens listed in Class 2 (See Appendix B), or are known to carry such pathogens must use P3 physical containment+an EK2 host-vector. When the potent toxin is not polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3+EK1 or P2+EK2. Species that produce potent toxins that affect invertebrates or plants: P2+EK2 or P3+EK1.

Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group unless it has been shown that those organisms used as the source of DNA do not contain these agents; in this case they may be placed in the following group.

2. The remainder of the species in this class: P2+EK1 or P1+EK2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of disease-causing microorganisms or must belong to a species that does not carry microorganisms causing disease in vertebrates or plants. If these conditions cannot be met, experiments must be done under P3+EK1 or P2+EK2 containment.

(f) *Plants.* P2 physical containment + an EK1 host-vector or P1+EK2. If the plant source carries a known pathogenic microorganism or makes a potent polypeptide toxin,[4] the containment must be raised to P3 physical containment + an EK2 host-vector. When the potent toxin is not polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3+EK1 or P2+EK2.

(g) *Cloning of viral genomes from eukaryotic cell DNA.* Efforts to isolate and amplify any part or all of an endogenous Retrovirus genome, or nucleotide sequences identified by their homology with a known Retrovirus genome, shall be carried out according to the

specifications for Retroviruses in Section b. Similar considerations apply to attempts to clone viral genomes from exogenously infected cells; containment conditions are those for the viral genome in question, as defined in Section b.

(2) *Prokaryotic DNA recombinants.* The minimum containment conditions consist of P2 physical containment+an EK1 host-vector or P1+EK2 and apply when the DNA is from those bacteria that have been extensively characterized as to the physiological, biochemical, and/or genetic properties and when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal. A description of the relevant properties of organisms which may meet these criteria should be submitted to the Office of Recombinant DNA Activities, NIH, for approval of this minimal containment level by the Director, NIH, on the recommendation of the Recombinant DNA Molecule Program Advisory Committee.

Experiments with DNAs from bacteria that are not extensively characterized require P2 physical containment+an EK2 host-vector or P3+EK1. Experiments with DNAs from pathogenic species (Class 2 and plant pathogens, See Appendix B) must use P3+EK2.

b. *Plasmids, bacteriophages, and other viruses.* Recombinants formed between a vector and some other plasmid or virus DNA have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombiants in *E. coli* K–12 hosts. They do not apply to other hosts in which the recombinants may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

(1) *Viruses of eukaryotes.*[5]—(a) *Viruses Of Warm-Blooded Vertebrates—1. DNA Viruses And DNA Transcripts Of Retrovirus Genomes.* P4 physical containment+an EK1 host-vector or P3+EK3 shall be used for DNA recombinants produced with the whole genome or in shotgun type experiments with unpurified segments thereof.

Containment can be reduced to P3+EK2 for clones shown to contain less than the complete potentially infectious viral genome, or for cloning purified[6] subgenomic segments. However, in both cases, if the cloned segment contains any portion of a viral gene region that is known or suspected to be responsible for neoplastic transformation, that portion must not be large enough to code for a functional polypeptide.

2. DNA Transcripts Of Other RNA Virus Genomes. In the case of RNA viruses with non-segmented genomes, containment conditions are as outlined in the preceding Section.

In the case of RNA viruses with segmented genomes, the virtual impossibility of synthesizing a single DNA molecule containing the entire viral genetic information from the extracted RNA permits a lower level of containment for shotgun type experiments. P3 physical containment+an EK2 host-vector may be used.

(b) *Viruses Of Cold-Blooded Vertebrates.—1. DNA Viruses And DNA Transcripts Of Retrovirus Genomes.* P4 physical containment +an EK1 hostvector or P3+EK2 shall be used for DNA recombinants produced with the whole genome or in shotgun type experiments with unpurified segments thereof.

Containment can be reduced to P2+EK2 for clones shown to contain less than the complete, potentially infectious viral genome or for cloning purified[6] subgenomic segments.[6]However, in both cases, if the cloned segment contains any portion of a viral gene region that is known or suspected to be responsible for neoplastic transformation, that portion must not be large enough to code for a functional polypeptide.

2. DNA transcripts of other RNA virus genomes. In the case of RNA viruses with non-segmented genomes, containment conditions are as outlined in the preceding Section. In the case of RNA viruses with segmented genomes, the virtual impossibility of synthesizing a single DNA molecule containing the entire viral genetic information from the extracted RNA permits a lower level of containment for shotgun type experiments. P2 physical containment + an EK2 host-vector may be used.

(c) *Viruses of invertebrates and protozoa.—1. Baculoviruses which have been registered by the Environmental Protection Agency as pesticides.*[7] For cloning all or part of the viral genome: P1 physical containment + an EK2 host-vector or P2 + EK1.

2. Other viruses. Same containment levels as for viruses of cold-blooded vertebrates.

(d) *Viruses of plants.* P3 physical containment+an EK1 host-vector or P2+EK2 conditions shall be used to form DNA recombinants that include all or part of the genome of a plant virus.

(2) *Eukaryotic organelle DNAs.* The containment conditions given below apply only when the organelle DNA has been purified[8] from isolated organelles. Mitochondrial DNA from primates: P3 physical containment+an EK1 host-vector or P2 + EK2. Mitochondrial or chloroplast DNA from other eukaryotes: P2+EK1. Otherwise, the conditions given under shotgun experiments apply.

(3) *Prokaryotic plasmid and phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages.

c. *Lowering of containment levels for characterized or purified DNA preparations and clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone, made from a random assortment of DNAs, has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

(1) *Purified DNA other than plasmids, bacteriophages, and other viruses.* The formation of DNA recombinants from cellular DNAs that have been purified[8] by physical and chemical techniques and which are free of harmful genes can be carried out under lower containment conditions than used for the corresponding shotgun experiment.[9] In general, the containment may be decreased one step in physical containment (P4→P3→P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (EK3→EK2→EK1) while maintaining the specified physical containment—provided that the new containment condition is not less than that specified below for characterized clones from shotgun experiments.

The institutional biohazards committee (IBC) may approve such a reduction. The IBC must notify the Office of Recombinant DNA Activities (ORDA) in writing of all such actions.

(2) *Characterized clones of DNA recombinants.* When a cloned DNA recombinant has been rigorously characterized and there is sufficient evidence that it is free of harmful genes,[3] experiments involving this recombinant DNA may be carried out under lower containment conditions.

(a) Institutional biohazards committees (IBCs) may give approval for a single step reduction in physical or biological containment on receipt of evidence of characterization of the clone and its probable freedom from harmful genes. The IBC must notify the Office of Recombinant DNA Activities (ORDA) in writing of all such actions.

(b) Reduction of containment levels by more than one step will require prior approval by ORDA.

2. *Experiments with other prokaryotic host-vectors.* Host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and prokaryotic DNA from non-pathogenic prokaryotes which do not produce polypeptide toxins[4] (i.e., organisms which can be cloned into EK1 hosts under P2 conditions).

Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. Experiments with DNAs from eukaryotes (and their plasmids or viruses) will generally follow the criteria for the corresponding experiments with *E. coli* K–12 host-vectors if the major habitats of the given host-vector overlap those of *E. coli*. The habitats of other host-vector systems should also be considered in relation to containment.

3. *Experiments with eukaryotic host-vectors.*—a. *Vertebrate host-vector systems.* Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by humans; thus vectors that have little or no ability to replicate in human cells should be chosen. Also, a recombinant virus should not inadvertently pose a threat to any species. Prior to its use as a vector in a vertebrate host cell system, a DNA molecule needs to display the following properties:

(i) It shall not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) If the DNA is of viral origin, the virus should not exhibit significant pathogenicity in its natural biology, and the range of species in which the virus replicates efficiently should be narrow.

(iii) Its functional anatomy shall be known—that is, there should be a clear idea of the location within the molecule of:

(a) The sites at which DNA synthesis originates and terminates.

(b) The sites that are cleaved by restriction endonucleases.

(c) The template regions for the major gene products.

(iv) It must be defective when carrying an inserted DNA segment; that is, propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome. Subject to the limitation in (i) above, it is not required in all cases that the recombinant be derived from a defective vector molecule; defectiveness resulting from inactivation of an essential gene by the inserted DNA sequence is sufficient. If possible, the helper should either: (a) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector to that particular cell line), or (b) con-

sist of a defective genome or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation. However, this is not a requirement.

In the case of very short insertions, i.e., of less than 50 base pairs, it is not necessary that the recombinant be defective. However, three requirements must be met. First, the inserted sequence must be either (a) a product of in vitro synthesis; or (b) a sequence, or segment of a sequence, which has been purified [6] by chemical means or by cloning, and which does not code for a toxic product. Second, it must not be of viral origin. Third, it must be confirmed that the virus containing the inserted segment has not increased in its ability to replicate in human cells in tissue culture.

Currently the only vertebrate viral DNAs that can be considered as meeting these requirements are the genomes of polyoma virus, SV40, and Adenovirus types 2 and 5.

(1) *Polyoma virus.* (a) Defective or intact polyoma virus genomes, with appropriate helper, if necessary, can be used in P3 conditions to propagate DNA sequences from:

Bacteria of Class 1 or Class 2 (See Appendix B), or their phages or plasmids, except for species of bacteria that produce potent polypeptide toxins.[4]

Eukaryotic organisms that do not produce potent polypeptide toxins.[4] Whenever there is a choice, it is urged that mouse cells, derived preferably from embroyos, be used as the source of eukaryotic DNA. Polyoma virus is a mouse virus and recombinant DNA molecules containing both viral and cellular sequences are already known to be present in virus stocks grown at a high multiplicity. Thus, recombinants formed in vitro between polyoma virus DNA and mouse DNA are presumably not novel from an evolutionary point of view.

(b) Polyoma genomes that are unconditionally defective by virtue of a deletion in an essential gene region may be used, with appropriate helper, in P3 conditions to propagate, in mouse cell cultures, subgenomic segments of Class 1 or Class 2 viruses (See Appendix B) which do not replicate in mouse cells: *Provided*, That it is clear from the nature of the DNA segments to be combined that there is no mechanism whereby a nondefective virus could be generated. Viral DNA preparations used for ligation should contain less than 1% contamination with intact genomes.

It must be verified that the defective-virus/helper-virus system has not been altered by the inserted DNA so as to replicate in human tissue culture cells.

(2) *SV40 and adenovirus types 2 and 5.* (a) Genomes of SV40 and Ad. 2 and 5 which are unconditionally defective by virtue of a deletion in an essential gene region may be used, with helper, as vectors for DNA molecules of the types listed under Polyoma virus (See (1) above). P4 conditions are required. Viral DNA preparations used for ligation should contain less than 1% contamination with intact genomes.

(b) Such experiments may be carried out in P3 conditions if the inserted DNA segment is (i) a purified [6] segment of prokaryotic DNA lacking toxigenic genes, or (ii) a segment of eukaryotic DNA whose gene product has been identified, which does not code for a toxic product, and which has been previously cloned in a prokaryotic host-vector system, or in P4 conditions as in (a), above. In the case of SV40, if helper is used, it shall be confirmed that the defective-virus/helper-virus system does not reproduce significantly more viral DNA or progeny virus in human cells in tissue culture than does SV40, following infection at a multiplicity of infection of one or more helper SV40 viruses per cell; P3 conditions may be used for this testing.

(c) A viral DNA molecule which is unconditionally defective by virtue of a deletion in the capsid genes and which is capable of autonomous replication (analogous to a plasmid) can be used as a vector for propagating DNA from Class 1 agents (See Appendix B) in established cell lines. P3 conditions can be used for shotgun type experiments: *Provided*, That the vector is known to be free of the complete viral genome by virtue of having been propagated serially in its absence. Otherwise, P4 containment conditions must be used.

(d) Recombinant DNA molecules consisting of SV40 or Ad. 2 or 5 DNA plus subgenomic sequences from eukaryotic organisms or Class 1 or Class 2 agents (See Appendix B) may be used to transform nonpermissive cells under P3 conditions. The recombinant molecule must be defective, and it must be demonstrated that no infectious virus particles are being produced; rescue of SV40 from such transformed cells by co-cultivation or transfection techniques must be carried out in P4 conditions.

b. *Invertebrate host-vector systems: Pesticide baculoviruses.* Baculoviruses which have been registered by the Environmental Protection Agency for commercial dissemination as biological pesticides have been suggested for use as cloning vectors. Two viruses are presently registered, the *Heliothis zea* baculovirus for control of the cotton bollworm and tobacco budworm, and the *Orygia pseudotsugata* baculovirus for control of the Douglas-fir tussock moth.[7] These viruses have a host range which is restricted to their natural host(s), which are serious insect pests; they have been extensively tested for pathogenicity for many species of vertebrates and invertebrates, with completely negative results (28); they replicate best and cause natural disease only in the larval stage of the insect, thus simplifying containment; they have no taxonomic relatives among viruses of vertebrates; and tissue culture systems are available. From available data, these properties appear to offer great safety features. However, much still needs to be learned. Particularly needed is information on the nature of the host range specificity, and on the infectivity and persistence of the viral DNA in vertebrate and invertebrate cell cultures.

When such background information is available, and if it confirms the narrow host range specificity, a baculovirus vector may be used for cloning DNA segments derived from the host insect, from another Environmental Protection Agency registered baculovirus, or from an EK1 bacterium using P2 physical containment. During the exploratory phases of this work it is not envisioned that the cloning of other classes of DNA will need to be studied.

c. *Plant host-vector systems.* For cells in tissue cultures, seedlings, plant parts (e.g., tubers, stems, fruits, and detached leaves) or whole mature plants of small species (e.g., *Arabidopsis*) the descriptions of P1–P4 physical containment conditions that have been previously specified are adequate. However, work with most whole plants pose additional problems. The greenhouse facilities approximating P2 laboratory physical containment conditions can be provided by: (i) Insect-proof greenhouses, (ii) appropriate sterilization of contaminated plants, pots, soil, and run-off water, and (iii) adoption of the other standard practices for microbiological work. P3 physical containment can be sufficiently approximated by confining the operations with whole plants to growth chambers like those used for work with radioactive isotopes, provided that (i) such chambers are modified to produce a negative pressure environment with the exhaust air appropriately filtered, (ii) other operations with infectious materials are carried out under the specified P3 conditions, and (iii) growth chambers are routinely fumigated and only used in insect-proof rooms in order to guard against inadvertent insect transmission of recombinant DNA. The P2 and P3 conditions specified earlier are therefore extended to include these cases for work on higher plants.

Whole plants or plant parts that cannot be adequately contained shall not be used as hosts for recombinant DNA experiments at this time.

Organelle or plasmid DNAs or DNAs of viruses of restricted host range may be used as vectors. In general, criteria for selecting viruses similar to those given in the preceding section on animal systems are to apply to plant systems.

DNA recombinants formed between the initial moderately contained vectors, and DNA from cells of species in which the vector DNA can replicate, require P2 physical containment. However, if the source of the DNA is itself pathogenic or known to carry pathogenic agents, or to produce products dangerous to plants, or if the vector is an unmodified virus, the experiments shall be carried out under P3 conditions.

Experiments on recombinant DNAs formed between the above vectors and DNAs from other species may also be carried out under P2 containment if that DNA has been purified [6] and determined not to contain harmful genes. Otherwise, the experiments shall be carried out under P3 conditions if the source of the inserted DNA is not itself a pathogen, or known to carry such pathogenic agents, or to produce harmful products—and under P4 conditons if these conditions are not met.

The development and use af host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit a decrease of one step in the physical containment specified above (P4→P3→P2→P1).

d. *Fungal or similar lower eukaryotic host-vector systems.* The Containment criteria for experiments on recombinant DNAs using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. Therefore, the containment guidelines given for experiments with prokaryotic host-vectors (Sections III B–1 and –2) provide adequate direction for experiments with these lower eukaryotic host-vectors. This is particularly true at this time since the development of these host-vectors is presently in the speculative stage.

4. *Complementary DNAs.* Where applicable, cDNAs (i.e., complementary DNAs) synthesized in vitro from cellular or viral RNAs are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under B.1.a., shotgun experiments; cDNAs formed from viral RNAs are included under B.1.b; cDNAs formed from purified and characterized RNAs are included under B.1.c.; etc. Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

5. *Handling recombinant DNA molecules.* Preparations of recombinant DNA molecules which do not contain viable organisms should be handled with good microbiological techniques equivalent to those used in a P1 laboratory. All recombinant DNAs should, however, be inactivated prior to disposal.

IV. ROLES AND RESPONSIBILITIES

Safety in research involving recombinant DNA molecules depends upon how the research team applies these guidelines. Motivation and critical judgment are necessary, in addition to specific safety knowledge, to ensure protection of personnel, the public, and the environment.

The Guidelines given here are to help the principal investigator determine the nature of the safeguards that should be implemented. These Guidelines will be incomplete in some respects because all conceivable experiments with recombinant DNAs cannot be anticipated. Therefore, they cannot substitute for the investigator's own knowledgeable and discriminating evaluation. Whenever this evaluation calls for an increase in containment over that indicated in the Guidelines, the investigator has a responsibility to institute such an increase. In contrast, the containment conditions called for in the Guidelines may not be decreased without review and approval at the institutional and/or NIH levels.

The following roles and responsibilities define an administrative framework in which safety is an essential and integrated function of research involving recombinant DNA molecules.

A. *Principal investigator.* Each principal investigator shall have had adequate training in good microbiological techniques. The principal investigator has the primary responsibility for: (i) Determining the real and potential biohazards of the proposed research, (ii) determining the appropriate level of biological and physical containment, (iii) selecting the microbiological practices and laboratory techniques for handling recombinant DNA materials, (iv) preparing procedures for dealing with accidental spills and overt personnel contamination, (v) securing approval of the proposed research prior to initiation of work, (vi) submitting information on purported HV2 and HV3 systems to the NIH Recombinant DNA Molecule Program Advisory Committee and making the strains available to others, (vii) reporting to the institutional biohazards committee and the NIH Office of Recombinant DNA Activities new information bearing on the Guidelines, such as technical information relating to hazards and new safety procedures or innovations, (viii) applying to the institutional biohazards committee and/or to the NIH Office of Recombinant DNA Activities for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, and (ix) packaging and distributing organisms and viruses containing recombinant DNA in accordance with the specifications set forth in Section II., C. (Shipment).

Before work is begun, the principal investigator is responsible for: (i) Making available to program and support staff copies of the approved protocols that describe the biohazards and the precautions to be taken, (ii) advising the program and support staff of the nature and assessment of the real and potential biohazards, (iii) instructing and training this staff in the practices and techniques required to ensure safety, and in the procedures for dealing with accidentally created biohazards, and (iv) informing the staff of the reasons and provisions for any advised or requested precautionary medical practices, vaccinations, or serum collection.

During the conduct of the research, the principal investigator is responsible for: (i) Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed, (ii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities, the institutional biological safety officer (where applicable), and the institutional biohazards committee any serious or extended illness of a worker or any accident that results in (a) inoculation of recombinant DNA materials through cutaneous penetration, (b) ingestion of recombinant DNA materials, (c) probably inhalation of recombinant DNA materials following gross aerosolization, or (d) any incident causing serious exposure to personnel or danger of environmental contamination, (iii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities, the institutional biological safety officer (where applicable), and the institutional biohazards committee any significant problems pertaining to operation and implementation of biological and physical containment safety practices and procedures, or equipment or facility failure, (iv) correcting work errors and conditions that may result in the release of recombinant DNA materials, and (v) ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.).

B. *Institution.* Since in almost all cases NIH grants are made to institutions rather than to individuals, all the responsibilities of the principal investigator listed above are the responsibilities of the institution under the grant, fulfilled on its behalf by the principal investigator. In addition, the institution is responsible for establishing an institutional biohazards committee * to: (i) Advise the institution on policies, (ii) create and maintain a central reference file and library of catalogs, books, articles, newsletters, and other communications as a source of advice and reference regarding, for example. the availability and quality of the safety equipment, the availability and level of biological containment for various host-vector systems, suitable training of personnel, and data on the potential biohazards associated with certain recombinant DNAs, and (iii) develop a safety and operations manual for any P4 facility maintained by the institution and used in support of recombinant DNA research.

Each institution has the responsibility for ensuring that research personnel have been adequately trained in, and consistently employ, good microbiological technique. The institution shall certify to the NIH on applications for research support and annually thereafter, that facilities, procedures, and practices and the training and expertise of the personnel involved have been reviewed and approved by the institutional biohazards committee.

Each institution shall also determine the necessity of various medical procedures for research personnel, arrange for these necessary procedures, and regularly review the results. For example, such procedures might include pre-employment or pre-assignment history and physical examination to detect relevant medical situations, such as steroid therapy, immunosuppression, pregnancy, gastrointestinal disorders, including achlorhydria, or stomach or bowel resection or disorders which may require the use of antibiotics or large doses of antacids. Individuals receiving antibiotics should not work with prokaryotic hos-vector systems during therapy nor for seven days thereafter. The obtaining of one or more serum samples for analysis or storage may also be indicated, as may specific immunizations. Periodic examinations subsequently, as well as following any accidental exposure, may also be wise, as may be the maintenance by the institution of a registry of individuals involved in recombinant DNA research.

The biohazards committee must be sufficiently qualified through the experience and expertise of its membership and the diversity of its membership to ensure respect for its advice and counsel. Its membership should include individuals from the institution or consultants, selected so as to provide a diversity of disciplines relevant to recombinant DNA technology, biological safety, and engineering. In addition to possessing the professional competence necessary to assess and review specific activities and facilities, the committee should possess, or have available to it, the competence to determine the acceptability of its findings in terms of applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. The committee shoud, therefore, include persons whose concerns are in these areas.

Minutes of the meetings should be kept and made available for public inspection. The institution is responsible for reporting names of and relevant background information on the members of its biohazards committee to the NIH.

In addition, each institution in which recombinant DNA research at a P3 or P4 containment level is being conducted shall designate a biological safety officer. The safety officer, who may be a member of the institutional biohazards committee, shall be answerable to the responsible officer of the institution, and shall work in close cooperation with the institutional biohazards committee. The officer's duties shall include, but not be limited to, the following: (i) Verifying that all research personnel have been adequately trained in good microbiological techniques; (ii) ensuring through periodic inspections that laboratory safety standards are rigorously followed; (iii) investigating all laboratory accidents; (iv) reviewing the results of histories, physical examinations, and monitoring of laboratory personnel; and (v) providing advice on laboratory security.

C. *NIH initial review groups (study sections)*—The NIH study sections, in addition to reviewing the scientific merit of each grant application involving recombinant DNA molecules, are responsible for: (i) Making an independent evaluation of the containment levels required for the proposed research by these Guidelines, (ii) determining whether the proposed physical containment safeguards certified by the institutional biohazards committee are adequate, (iii) determining whether the proposed biological containment safeguards are adequate, (iv) referring to the NIH Recombinant DNA Molecule Program Advisory Committee or the NIH Office of Recombinant DNA Activities those problems pertaining to assessment of biohazards or safeguards determination that cannot be resolved by the study sections.

The membership of the study sections will be selected in the usual manner. Biological safety expertise, however, will be available to the study sections for consultation and guidance.

D. *NIR Recombinant DNA Molecule Program Advisory Committee*—The Recombi-

nant DNA Molecule Program Advisory Committee advises the Secretary, Department of Health, Education, and Welfare; the Assistant Secretary for Health, Department of Health, Education, and Welfare; and the Director, National Institutes of Health, on a program for the evaluation of potential biological and ecological hazards of recombinant DNAs, on the development of procedures which are designed to prevent the spread of such molecules within human and other populations, and on guidelines to be followed by investigators working with potentially hazardous recombinants.

The NIH Recombinant DNA Molecule Program Advisory Committee has responsibility for: (i) Recommending revisions of the Guidelines to be followed by investigators working with DNA recombinants, (ii) receiving information on proposed HV1, HV2 (EK2), and HV3 (EK3) host-vector systems, determining whether the proposed systems meet the established criteria, and recommending to the Director, NIH, the certification of systems which meet the criteria, and (iii) resolving questions concerning potential biohazards and adequacy of containment if the NIH Office of Recombinant DNA Activities so requests.

The Committee shall have the authority to recommend to the Director, NIH, exemptions from specific requirements of the Guidelines, when such exemptions are justified by exceptional circumstances. For example, the committee may recommend exemptions from the containment requirements of the Guidelines for committee-approved risk-assessment studies. Each request for such an exemption must be accompanied by adequate documentation, which shall include clear and convincing evidence of the need for the particular risk-assessment study. The committee may also recommend exemptions for specific experiments which fall within the six categories of "experiments that are not to be performed." In making such recommendations for exceptions, weight will be given both to scientific and societal benefits and to potential risks.

E. *NIH.* NIH staff has responsibility for: (i) Assuring that no NIH grants or contracts are awarded for recombinant DNA research unless they (a) conform to these Guidelines, (b) have been properly reviewed and recommended for approval, and (c) include a properly executed Memorandum of Understanding and Agreement, (ii) reviewing and responding to questions or problems or reports submitted by institutional biohazards committees or principal investigators, and disseminating findings, as appropriate, (iii) receiving and reviewing applications for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, (iv) referring items covered under (ii) and (iii) above to the NIH Recombinant DNA Molecule Program Advisory Committee, as deemed necessary, and (v) performing site inspections of all P4 physical containment facilities engaged in recombinant DNA research, and site inspections at other facilities as deemed necessary.

The Director, NIH, is responsible for overseeing implementation and for judicious and flexible interpretation of these Guidelines.

V. Footnotes

[1] In general, recombinant DNA formed between segments of eukaryotic viral DNA and any eukaryotic DNA (viral, cytrachromosomal) shall not be excluded from these Guidelines until such time as there is more information about the extent of naturally occurring recombinational events between these DNAs.

[2] Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency or high efficiency particular air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is used in three operational modes: (1) with an 8-inch high full width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm length rubber gloves. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Mich. A Class III cabinet is a closed front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through HEPA filters or incinerated before being discharged to the outside environment.

[3] The terms "characterized" and "free of harmful genes" are unavoidably vague. The following types of data should be considered: (a) The absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or host or be detrimental to humans, animals or plants); (b) the type(s) of genetic information on the cloned segment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable); (d) the genetic stability of the cloned fragment; and (e), any alterations in the biological properties of the vector and host.

[4] We are specifically concerned with potent toxins which could be produced by acquiring a single gene or cluster of genes.

[5] The containment requirements specified for DNA derived from viruses of eukaryotes were designed chiefly with two hypothetical risk mechanisms in mind. The first is the concern lest a bacterium carrying viral nucleic acid be able to produce virus infections; this is most easily envisioned as occurring from delivery of the viral nucleic acid into proximity with host cells. (Production of infectious virus within the host bacterium appears to be a most unlikely event, except possibly in the case of the smaller icosahedral viruses. However, further data are needed.) Obviously, this mechanism applies only when the complete viral genome is present, and containment conditions are highest when there is a possibility that the whole genome is present.

The second hypothetical risk mechanism is focused on viral oncogenesis. The chief component of the model is again delivery of the viral nucleic acid to host cells, with subsequent integration of the viral DNA into the genome of host cells. In some DNA virus systems, cell transformation can be produced by a segment of the viral genome; such segments are from the early region of the viral genome, and presumably code for products which affect regulation of DNA synthesis or function. Genes coding for the synthesis of virus capsid components have not been associated with oncogenesis. Consequently, containment conditions for subviral segments from DNA viruses are set higher for gene regions coding for nonstructural proteins, which are chiefly involved with DNA synthesis, than for genes for virion structural components.

With the exception of the Retroviruses (i.e., the family of viruses whose members possess the reverse transcriptase enzyme, and which includes a number of oncogenic viruses (Fenner, F., Classification and Nomenclature of Viruses; Intervirology, 7:1–116, 1976.)), RNA viruses have not been implicated in tumorigenesis. Further, the genes of RNA viruses analogous to those of DNA viruses which specify early functions and nonstructural protein synthesis tend to be specific for the RNA of that virus, and are not thought to affect cellular processes.

The concept of production by the host bacterium of a viral protein which would act from outside the cells as a carcinogenic substance is without experimental basis. Also, there is no instance of a highly toxic viral product. Production of disease by a viral infection requires the functioning of the entire virus; there is no basis for concern that a eukaryotic viral DNA segment might cause the host bacterium to produce a toxin.

It should be pointed out that the concern about risk from a microorganism carrying a viral insert is directed at those species that may come in contact with the host microorganism, and is not particularly directed at those species that the virus infects in nature.

[6] The DNA preparation is defined as "purified" if the desired DNA represents at least 99 percent (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

[7] Baculoviruses which have been registered to date include the *Heliothis zea* baculovirus, registration number 11273–17 (Jan. 1976) and the *Orgyia pseudotsugata* baculovirus, Registration number 27586–1 (Aug. 1976).

[8] The reason for lowering the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10^5-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

[9] In special circumstances, in consultation with the NIH Office of Recombinant DNA Activities, an area biohazards committee may be formed, composed of members from the institution and/or other organizations beyond its own staff, as an alternative when additional expertise outside the institution is needed for the indicated reviews.

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APPENDIX A

PACKAGING AND SHIPPING OF RECOMBINANT DNA MATERIALS

A. *Introduction.* Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all recombinant DNA materials will be packaged and shipped in containers that meet the requirements of these regulations and carrier tariffs.

B. *Packaging of recombinant DNA molecules.* Recombinant DNA molecules shall be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

C. *Packaging of host and vector organisms containing recombinant DNA molecules.* 1. Volume less than 50 ml. Material shall be placed in a securely closed, watertight container (primary container (test tube, vial, etc.)) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s).

Descriptions of this packaging method are given in Table I (see page A-7).

2. Volumes of 50 ml. or greater. Material shall be placed in a securely closed, watertight container (primary container) which shall be enclosed in a second, durable watertight container (secondary container). Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. A shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and the outer shipping container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of materials which may be enclosed within a single outer shipping contained should not exceed 4,000 ml.)

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages which comply with the regulations of the Department of Transportation (DOT) are given in Table II (see page A-8).

D. *Labeling of packages containing host and vector organisms which contain recombinant DNA molecules.* Material data forms, leters, and other information identifying or describing the material should be placed around the outside of the secondary container. The label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in paragraph (c)(4) of 42 CFR 72.25. In addition, if the materials to be shipped contain any portion of a plant pest (plant pathogens) which is so defined by the Department of Agriculture (USDA), the outer shipping container shall have affixed to it the shipping label furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents.

E. *Additional shipping requirements and limitations for host and vector organisms which contain recombinant DNA molecules.*— 1. *Domestic transportation.* Civil Aeronautics Board Rule No. 82 (Air Transport Association Restricted Articles Tariff 6-D) requires that a Shipper's Certificate, depicted below, be completed and affixed to all shipments which bear the Etiologic Agent/Biomedical Materials label required under the provisions of the Interstate Quarantine regulations (42 CFR 72.25(c)). The Certificate must be completed in duplicate and affixed to the outer shipping container.

This is to certify that the contents of this consignment are properly classified, described by proper shipping name and are packed, marked and labelled and are in proper condition for carriage by air according to all applicable carrier and government regulations. (For international shipments add "and to the IATA Restricted Articles Regulations".) This consignment is within the limitations prescribed for: PASSENGER AIRCRAFT/CARGO ONLY (cross out nonapplicable).

Number of Packages	Specify Each Article Separately (Proper Shipping Name)	Classification	Net Quantity per Package
	ETIOLOGIC AGENT, n.o.s.	ETIO. AG.	

Shipper:

Date _____

(Signature of Shipper)

Shipments of materials exceeding 50 ml in volume are restricted, by DOT regulations, to transport by cargo only aircraft. When the volume of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on the Shipper's Certificate by crossing out "Passenger Aircraft."

When dry ice is used as a refrigerant an "ORA—Group A—Dry Ice Label" should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

2. *International transportation.* In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of these materials must have one or more of the following documents—depending on the country of destination:

(1) Parcel Post Customs Declaration (PS 2966) tag.

(2) Parcel Post Customs Declaration (PS 2966–A) label.

(3) International Parcel Post—Instructions Given by Sender (POD 2922) label.

(4) Dispatch note (POD 2972) tag.

(5) "Violet Label".

(6) Shipper's Certificate specified in the current International Air Transport Association Tariff. Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

NIH Guidelines

TABLE I

Description of Packages for Material in Volumes less than 50 ml.

Volume (ml)	Primary Container	Packing	Secondary Container	Packing	Outer Shipping d/ Container
15 max.	Sealed vial(s) or small glass test tube, screw cap* or stopper, taped	a/	Metal can 1" diam. x 3" O.D. metal screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 1-1/2" diam. x 7 to 7-1/2" O.D.
50 or less	One 20 x 150 mm test tube, taped* stopper or multiple small vials	a/	Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 3-1/4" diam. x 7 to 7-1/2" O.D.
50 or less	Plastic* screw cap* bottle or Pyrex glass with skirt rubber stopper	a/	Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 3-1/4" diam. x 7 to 7-1/2" O.D.
50 or less	Multiple watertight vials or * tubes, taped stoppers	a/	One or more friction-seal tin cans b/ 306 x 400 or larger	c/	Fiberboard box

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 700 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16".

c/ None required, but with the 306 x 400 cans or larger cans use sufficient nonparticulate shock-absorbent material to prevent rattling.

d/ If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the secured (original) outer shipping container. A leak proof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

HHS Guidelines

TABLE III

Description of Packages for Material in Volumes of 50 ml or greater

Volume (ml)	Primary Container	Packing	Secondary Container	Packing: With Refrigerant	Packing: Without Refrigerant	Outer Shipping Container: With Refrigerant	Outer Shipping Container: Without Refrigerant
51 to 100 ml	Plastic* or Pyrex glass screw cap* bottle; rubber or skirt rubber stopper, taped*	a/	Consists of metal container & outer container specified in Table III	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	Corrugated fiberboard or cardboard box, taped shut
100 max.	One 100 ml plastic* screw cap* narrow neck bottle or Pyrex glass, taped*	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PSJ type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PSJ tape
200 max.	Two 100 ml plastic* screw cap* bottles or Pyrex glass, taped	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PSJ type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PSJ tape
250 max.	One 250 ml, plastic* narrow mouth screw cap* bottle or Pyrex glass skirted rubber stopper, taped*	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PSJ type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PSJ tape
500 max.	Two 250 ml plastic* screw cap* bottles or Pyrex glass bottles, taped*	a/	2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PSJ tape.
500 max.	500 ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500 ml plastic* bottle, narrow or wide mouth, screw cap*, taped	a/	No. 12 crimp seal tin can 603 x 810 2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PSJ tape. For the No can a cardboard box is ok taped shut

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 708 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16".

c/ Shock absorbent material, in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.

APPENDIX B

CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

I. Classification of Etiologic Agents on the Basis of Hazard (1)

A. Class 1 agents

All bacterial, parasitic, fungal, viral, rickettsial and chlamydial agents not included in higher classes.

B. Class 2 agents

1. Bacterial agents:

Actinobacillus—all species except *A. mallei*, which is in Class 3.
Arizona hinshawii—all serotypes.
Bacillus anthracis.
Bordetella—all species.
Borrelia recurrentis, B. vincenti.
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani.
Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale.
Diplococcus (Streptococcus) pneumoniae.
Erysipelothrix insidiosa.
Escherichia coli—all enteropathogenic serotypes.
Haemophilus ducreyi, H. influenzae.
Herellea vaginicola.
Klebsiella—all species and all serotypes.
Leptospira interrogans—all serotypes.
Listeria—all species.
Mima polymorpha.
Moraxella—all species.
Mycobacteria—all species except those listed in Class 3.
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 5.
Neisseria gonorrhoeae, N. meningitidis.
Pasteurella—all species except those listed in Class 3.
Salmonella—all species and all serotypes.
Shigella—all species and all serotypes.
Sphaerophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema carateum, T. pallidum, and T. pertenue.
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus.*

2. Fungal agents:

Actinomycetes (including *Nocardia* species and *Actinomyces* species and *Arachnia propionica*).
Blastomyces dermatitidis.
Cryptococcus neoformans.
Paracoccidioides brasiliensis.

3. Parasitic agents:

Endamoeba histolytica.
Leishmania sp.
Naegleria gruberi.
Toxoplasma gondii.
Toxocara canis.
Trichinella spiralis.
Trypanosoma cruzi.

4. Viral, rickettsial, and chlamydial agents:

Adenoviruses—human—all types.
Cache Valley virus.
Coxsackie A and B viruses.
Cytomegaloviruses.
Echoviruses—all types.
Encephalomyocarditis virus (EMC).
Flanders virus.
Hart Park virus.
Hepatitis-associated antigen material.
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4.
Corona viruses.
Influenza viruses—all types except A/PR8/34, which is in Class 1.
Langet virus.
Lymphogranuloma venereum agent.
Measles virus.
Mumps virus.
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1.
Polioviruses—all types, wild and attenuated.
Poxviruses—all types except *Alastrium, Smallpox, Monkey pox*, and *Whitepox*, which, depending on experiments, are in Class 3 or Class 4.

Rabies virus—all strains except *Rabies street virus*, which should be classified in Class 3 when inoculated into carnivores.
Reoviruses—all types.
Respiratory syncytial virus.
Rhinoviruses—all types.
Rubella virus.
Simian viruses—all types except *Herpesvirus simiae* (*Monkey B virus*) and *Marburg virus*, which are in Class 4.
Sindbis virus.
Tensaw virus.
Turlock virus.
Vaccinia virus.
Varicella virus.
Vole rickettsia.
Yellow fever virus, 17D vaccine strain.

C. Class 3 agents

1. Bacterial agents:
*Actinobacillus mallei.**
Bartonella—all species.
Brucella—all species.
Francisella tularensis.
Mycobacterium avim, M. bovis, M. tuberculosis.
Pasteurella multocide type B ("buffalo" and other foreign virulent strains*).
*Pseudomonas pseudomallei.**
Yersenia pestis.
2. Fungal agents:
Coccidioides immitis.
Histoplasma capsulatum.
Histoplasma capsulatum var. *duboisii.*
3. Parasitic agents: *Schistosoma mansoni.*
4. Viral, rickettsial, and chlamydial agents:
Alastrim, Smallpox, Monkey pox, and *Whitepox*, when used *in vitro.*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory).
Dengue virus, when used for transmission or animal inoculation experiments.
Lymphocytic chorimeningitis virus (LCM).
Psittacosis-Ornithosis-Trachoma group of agents.
Rabies street virus, when used in inoculations of carnivores (See Class 2).
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments.
*Vesicular stomatitis virus.**
Yellow fever virus—wild, when used *in vitro.*

* USDA permit also required for import or interstate transport.

D. Class 4 agents

1. Bacterial agents: None.
2. Fungal agents: None.
3. Parasitic agents: None.
4. Viral, rickettsial, and chlamydial agents:

Alastrim, Smallpox, Monkey pox, and *Whitepox*, when used for transmission or animal inoculation experiments.
Hemorrhagic fever agents, including *Crimean hemorrhagic fever* (*Congo*), *Junin*, and *Muchupo* viruses, and others as yet undefined.
Herpesvirus simiae (Monkey B virus).
Lassa virus.
Marburg virus.
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever*, and *Central European encephalitis viruses.*
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments.
Yellow fever virus—wild, when used for transmission or animal inoculation experiments.

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (2)

A. Low risk oncogenic viruses

Rous Sarcoma.
SV–40.
CELO.
A7–SV40,
Polyoma.
Bovine papilloma.
Rat mammary tumor.
Avian Leukosis.
Murine Leukemia.
Murine Sarcoma.
Mouse mammary tumor.
Rat Leukemia.
Hamster Leukemia.
Bovine Leukemia.
Dog Sarcoma.
Mason-Pfizer Monkey Virus.
Marek's.
Guinea Pig Herpes.
Lucke (Frog).
Adenovirus.
Shope Fibroma.
Shope Papilloma.

B. Moderate risk oncogenic viruses

Ad2–SV40.
FeLV.
HV Saimiri.
EBV.
SSV–1.
GaLV.
HV ateles.
Yaba.
FeSV.

III. Animal Pathogens (3)

A. Animal disease organisms which are forbidden entry into the United States by law (CDC Class 5 agent)

1. Foot and mouth disease virus.

B. Animal disease organisms and vectors which are forbidden entry into the United States by USDA Policy (CDC Class 5 agents)

African horse sickness virus.
African swine fever virus.
Besnoitia besnoiti.
Borna disease virus.
Bovine infectious petechial fever.
Camel pox virus.
Ephemeral fever virus.
Fowl plague virus.
Goat pox virus.
Hog cholera virus.
Louping ill virus.
Lumpy skin disease virus.
Nairobi sheep disease virus.
Newcastle disease virus (Asiatic strains).
Mycoplasma mycoides (contagious bovine pleuropneumonia).
Mycoplasma agalactiae (contagious agalactia of sheep).
Rickettsia ruminatium (heart water).
Rift valley faver virus.
Rinderpest virus.
Sheep pox virus.
Swine vesicular disease virus.
Teschen disease virus.
Trypanosoma vivax (Nagana).
Trypanosoma evansi.
Theileria parva (East Coast fever).
Theileria annulata.
Theileria lawrencei.
Theileria bovis.
Theileria hirci.
Vesicular exanthema virus.
Wesselsbron disease virus.
Zyonema farciminosum (pseudofarcy.)

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2. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790.
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